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ORIGINAL ARTICLE

Individualised adjuvant immunotherapy with neoantigen-reactive T cells for gastric signet-ring cell carcinoma

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Abstract

Objectives. The signet-ring cell carcinoma (SRCC) of the stomach is highly invasive. Patients with stage III gastric SRCC usually experience tumor recurrence within 2 years after radical surgery. Unfortunately, there is no effective treatment to postpone recurrence following adjuvant chemotherapy. Our study aimed to explore the safety and efficacy of neoantigen-reactive T lymphocytes (NRTs) in patients with stage III gastric SRCC. **Methods.** The study included 20 patients with stage III gastric SRCC who received radical surgery and adjuvant chemotherapy. Following the adjuvant chemotherapy, they underwent treatment with a range of one to four cycles of personalised neoantigen-reactive T cells. The primary endpoint was the median progression-free survival (mDFS). The secondary endpoint was safety and immune responses. The median duration of follow-up was 41 months (95% CI: 39–42.9 months). **Results.** Our results showed that patients who received adjuvant neoantigen-reactive T-cell immunotherapy demonstrated a propensity towards prolonged disease-free survival (DFS) and overall survival (OS) in comparison to previous studies. The 2-year DFS and OS rates reached 73.7% and 95%, respectively, whereas the 5-year DFS and OS rates were 44% and 69%. The median DFS was 41 months (95% CI: 28.9–53.1 months) and the median OS was not reached. In addition, there was a significant increase in serum concentrations of IL-2, IL-4, IL-6, IL-10, TNF-α and IFN-γ after cell immunotherapy. The adverse reactions were mild. **Conclusion.** In conclusion, adjuvant immunotherapy with NRTs showed promising efficacy alongside a manageable safety profile.

Keywords: adoptive cell immunotherapy, gastric cancer, neoantigen-reactive T cells, signet-ring cell carcinoma

INTRODUCTION

Gastric cancer is one of the most common prevalent malignancies globally. There are 500 000 new gastric cancers in China annually. This accounts for 49% of the total worldwide incidence. Most of the cases were in advanced stages at the time of diagnosis, resulting in a five-year survival rate of less than 30%.[1,2] Previous studies reveal diverse histological subtypes with gastric cancer, with signet-ring cell carcinoma (SRCC) representing 15–28% of all stomach malignancies. This subtype distinctly differs in clinicopathological characteristics from other histological subtypes of gastric cancer.[3] It has the characteristics of high malignancy, poor differentiation, invasive and diffuse growth in the gastric wall, rapid progression and elusive early diagnosis.[4,5] While the early-stage prognosis of gastric SRCC surpasses that of other types, the prognosis deteriorates considerably in advanced stages. The median overall survival of stage III gastric SRCC was reported to be only 19.7 months.[6] Adjuvant therapy for stage III gastric SRCC is still controversial. Present gastric cancer guidelines advocate for combined chemotherapy as the standard treatment post D2 radical gastrectomy for stage III gastric cancer patients. However, many studies showed that gastric SRCC is resistant to chemotherapy, with higher proportions of signet-ring cells correlating to increased chemotherapy resistance.[7] Thus, the quest for novel treatment modalities beyond adjuvant chemotherapy has become an urgent imperative.

During 2015–2020, substantial advancements have been achieved in T-cell immunotherapy, particularly in hematologic malignancies. Moreover, its potential has been preliminarily explored in gastric cancer.[8,9] A study on advanced gastric cancer revealed that combining chemotherapy with T-cell immunotherapy prolonged PFS in patients with stage III gastric cancer. The 2-year PFS of the two groups was 62.5% and 25.7%, respectively. A subgroup analysis showed that immune cell therapy also exhibited positive outcomes for patients with poor differentiation disease. In cases where combined therapy was administered, the 2-year PFS was measured at 56.3%, surpassing the 28.6% PFS rate observed in patients subjected to chemotherapy alone.[10] Another study also showed that combined DC-CIK with S-1 plus cisplatin yielded favorable results in terms of both PFS and OS for patients with advanced gastric cancer.[11]

Plenty of preclinical and clinical studies have shown that neoantigens, arising from mutated genes, are optimal targets for T-cell immunotherapy.[12,13] However, how to effectively identify neoantigen-reactive T cells and apply them to cancer patients is still a challenge. In our previous study, we designed a shared neoantigen peptide library and provided a sequencing-independent pattern for timely and efficient identification of neoantigen. Furthermore, we have successfully identified neoepitopes using this library and induced neoantigen reactive T cells, and the antitumor efficiency of the induced neoantigen-reactive T cell was tested in patients with relapsed and refractory solid tumors.[14] As far as we know, no study has evaluated the antitumor efficiency of neoantigen-reactive T cells during the adjuvant therapy stage. Based on our previous study, a single-centre prospective clinical trial was carried out to assess the efficiency and safety of neoantigen-reactive T-cell immunotherapy for patients with stage III gastric SRCC after postoperative chemotherapy.

RESULTS

Characteristics of patients

From April 2016 to January 2019, we enrolled a total of 20 patients (14 males and six females) with stage III (stage IIIA, $n = 11$; stage IIIB, $n = 5$; stage IIIC, $n = 4$) gastric SRCC. The characteristics of the enrolled patients are shown in Table 1. The median age of the enrolled patients was 49 years (range: 25–77 years). Among the enrolled individuals, 17 patients received six cycles of postoperative standard chemotherapeutic regimens. These regimens included docetaxel and S1 ($n = 11$) or oxaliplatin and S1 ($n = 6$). However, as a result of intolerance to chemotherapy toxicities, three patients only received three cycles of chemotherapy (docetaxel and S1, $n = 2$; oxaliplatin and S1, $n = 1$). Following the completion of the last chemotherapy cycle, patients underwent evaluation after an interval of 2.02 months (ranging from 0.7 to 4.03 months). Subsequently, patients who displayed no signs of recurrence received 1–4 cycles of NRT (Neoantigen-reactive T cell) treatment, with the number of cycles as follows: one cycle ($n = 5$), two cycles ($n = 0$), three cycles ($n = 3$) and four cycles ($n = 12$). Each NRT treatment involved an infusion containing an average of 8.3×10^9 cells (ranging from 3.2×10^9 to 2.2×10^{10} cells). The interval between successive NRT infusions was about 6 weeks. Among the participants, 12 patients completed four cycles of

Table 1. Characteristics of the enrolled patients

Patient No.	Gender	Age (years)	Stage	Chemotherapy regimen	Cycles of Chemo	HLA type	Cycles ofNRT	Peptide
1	M	33	IIIA	Oxa + S	6	2402	4	PIK3CA-(H1047L) p1038-1047
2	M	57	IIIB	Oxa + S1	6	1101	1	TP53-(G245D) p240-248 KRAS-(G12R) p08-16
3	M	29	IIIA	Oxa + S1	3	1101	3	KRAS-(G12R) p07-16 TP53-(G245S) p240-248 KRAS-(G12V) p08-16 TP53-(E285K) p283-291
4	M	60	IIIA	Oxa + S1	6	0201	4	EBV-LMP2a356 KRAS-(G12C) p05-14 KRAS-(G13S) p05-14
5	F	51	IIIA	Doc + S1	3	1101	4	CDH1-(G441Afs*14)p443-449 CDH1-(G441Afs*14)p437-445 CDH1-(G441Afs*14)p434-448
6	M	25	IIIA	Doc + S1	6	0201/1101	4	TP53-(G245D) p240-248 EBV-LMP2a340
7	M	60	IIIA	Oxa + S1	6	0201/2402	4	CTNNB1(S37C) p30-39 KRAS-(G12 V) p05-1 4
8	F	65	IIIA	Doc + S1	6	0201	3	TP53-(R248Q) p247-255 TP53-(R248Q) p245-254 TP53-(R248W) p245-254
9	M	50	IIIA	Oxa + S1	6	0201/1101	4	EBV-LMP2a426
10	M	39	IIIA	Oxa + S1	6	1101	3	KRAS-(G12S) p07-16 TP53-(G245S) p240-248
11	M	71	IIIA	Doc + S1	6	0201/1101	1	KRAS-(G12V) p07-16 TP53-(E285K) p283-291 TP53-(R248W) p245-254
12	M	55	IIIB	Doc + S1	6	1101	4	KRAS-(G12S) p08-16 CTNNB1(T41A) p41-49 BRAF(V600K) p591-600 TP53-(Y163C) p156-164
13	F	34	IIIA	Doc + S1	6	0201/1101	4	KRAS-(G12C) p05-14 EBV-LMP2-A11–340 ERBB2-(S310F) p303-312 TP53-(R248Q) p247-255
14	M	77	IIIB	Doc + S1	6	2402	1	EGFR-(T790M) p786-795
15	M	38	IIIB	Doc + S1	6	0201/1101	4	TP53-(R248W) p240-249 P1K3CA-(H1047 L) p1046-1054
16	M	54	IIIB	Doc + S1	6	0201/1101	4	FBXW7-(R465H) p456-465 FBXW7-(R505C) p496-505 EBV-LMP2-A11–340
17	M	31	IIIC	Doc + S1	6	0201/1101	4	EBV-LMP2a356 CMV.PP65(02)-495 KRAS-(G12A) p05-14 EBV-LMP2a426
18	F	51	IIIC	Doc + S1	3	0201	1	EBV-LMP2a356 EBV-LMP2a426 CMV.PP65(02)-495 FBXW7-(R465C) p456-465
19	F	43	IIIC	Doc + S1	6	2402	1	EBV-LMP2a-419 EGFR-(T790M) p786-795
20	F	62	IIIC	Doc + S1	6	0201	4	FBXW7-(R465H) p456-465 TP53-(R248Q) p247-255 NRAS-(Q61L) p52-61 NOTCH2-(A21T) p06-15

Doc, docetaxel; F, female; M male; Oxa, Oxaliplatin; S1, Tegafur, Gimeracil and Oteracil Potassium Capsules.

cell infusion, three patients underwent three cycles of cell infusion and five patients received only 1 cycle of cell infusion. Of the eight patients who dropped from the protocol before the fourth cycle of cell infusion, one discontinued as a result of rapid disease progression and others discontinued of their own will.

Phenotypic analysis of *in vitro* cultured peripheral blood mononuclear cells (PBMCs)

Peripheral blood mononuclear cells were isolated and cultured for 14 days to generate NRTs in our GMP-grade laboratory. Phenotype analysis was performed before and post cell culture. The results showed that the proportion of $CD3^+$ and $CD3^+CD8^+$ T-cell subsets increased after cell culture. In contrast, there was a reduction observed in $CD3^+CD4^+$ T cells and B lymphocytes. No difference was seen in the proportion of NK cells. The percentage of neoantigen-specific T cells was 5.8% (0.52–19.47%) post-culture, affirming the successful induction of neoantigen-specific T cells. The absolute number of neoantigen-specific T cells per infusion was 4.42×10^8 (4.46×10^7 to 1.41×10^9). It is noteworthy that, there's no significant change in the proportion of $CD3^+CD279^+$ T cells, implying that our *in vitro* cell culture procedure did not lead to T-cell exhaustion. Furthermore, we also tested memory markers on T cells and found that the proportion of central memory $CD3^+CD45RO^+CD62L^+$ T cells decreased while effector memory $CD3^+CD45RO^+CD62L^-$ T cells increased (Figure 1).

Survival analysis

The median follow-up duration was 41 months (95% CI: 39–42.9 months). At the end of the study, 7 (35%) of the 20 patients had tumor recurrence and five (25%) patients died of tumor (Figure 2a). As shown in Figure 2b, the median DFS was 41 months (95% CI: 28.9–53.1 months) and the median OS was not reached. The 2-year DFS and OS rates were 73.7% and 95%, respectively. The 5-year DFS and OS rates were 44% and 69%, respectively. Potential prognostic factors that influence the therapeutic effect of NRT therapy were also evaluated. Although statistically insignificant, patients who finished the protocol had a tendency for prolonged DFS (5-year DFS, 62.5 months *vs* 21.4 months, $P = 0.18$) and OS (5-year OS, 83.3 months *vs* 53.6 months, $P = 0.36$) (Figure 2c). We also evaluated the effect of infused cell proportion on OS. As shown in Figure 2d–f, patients infused with a higher proportion of $CD3^+$ (5-year OS, 88.9 months *vs* 45.7 months, $P = 0.15$), $CD3^+CD8^+$ T cells (5-year OS, 87.5 months *vs* 52.5 months, $P = 0.13$), and a lower proportion of $CD3^+CD4^+$ T cells (5-year OS, 87.5 months *vs* 56 months, $P = 0.15$), might experience greater benefits from our adjuvant cell immunotherapy, indicating that the antitumor effect of NRT therapy is primarily mediated by cytotoxic T cells.

Adverse events

Treatment-related adverse events were manageable, mainly grade 1 and 2, and no grade 3 or 4 adverse event was observed. The most common adverse events associated with cell therapy during the study were fever (grade 1, $n = 5$; grade 2, $n = 7$), fatigue (grade 1, $n = 6$) and nausea (grade 1, $n = 5$), which were caused by cell-related agents. Additionally, two patients experienced transient grade 1 elevation of alanine aminotransferase (ALT), which subsequently ameliorated following supportive treatment (Table 2).

Cytokines levels after treatment

Cytokine levels, including IL-2, IL-4, IL-6, IL-10, TNF-α and IFN-γ were investigated in blood samples prior to immunotherapy and before the fourth cycle of T-cell infusion respectively. The duration between pre-treatment and post-treatment samples was 4.02 months (range: 2.8–4.9 months). Post-immunotherapy samples were not available in patients who failed to complete four cycles of T-cell infusion. As shown in Figure 3, significant increases were observed in all tested cytokines subsequent to cell therapy.

DISCUSSION

Gastric SRCC represents a distinct pathological subtype of gastric cancer characterised by its limited adhesiveness, leading to a higher recurrence and metastasis rate. Adjuvant therapy for stage III gastric cancer largely depends on conventional chemotherapy, yet its efficacy remains contentious because of emerging evidence indicating that gastric SRCC might exhibit chemoresistance.[6] There're also clinical trials assessing postoperative radiotherapy in gastric SRCC, whereas the

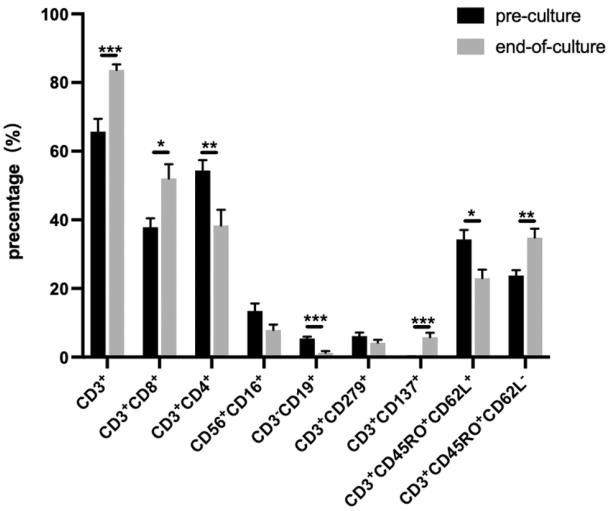

Figure 1. Peripheral blood T cell phenotype measurements before and after *in vitro* cell activation ($n = 20$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, Paired Student's *t*-test.

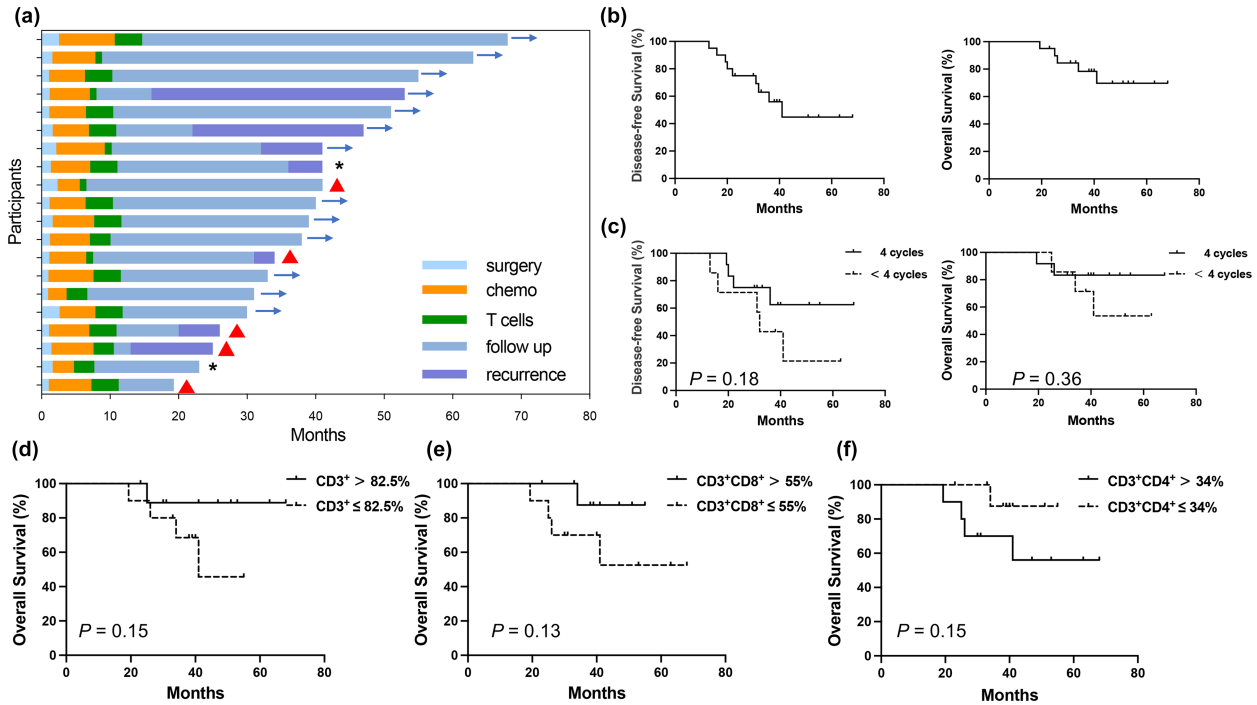

Figure 2. Survival analysis. Clinical event timeline for 20 patients from surgery until the time of data cutoff ($n = 20$) **(a)**. Arrows, people still alive at the time of the last follow-up. Triangle, people who died. *, missing follow-up. OS and DFS for all patients ($n = 20$) **(b)**. OS and DFS for patients who received four cycles of NRT ($n = 12$) and less than four cycles of NRT ($n = 8$) (log-rank test) **(c)**. Patients infused with a higher proportion of $CD3^+$ ($n = 10$) **(d)**, $CD3^+CD8^+$ ($n = 10$) **(e)** cells, and a lower proportion of $CD3^+CD4^+$ T cells ($n = 10$) **(f)** tend to have a longer survival time (log-rank test).

effectiveness also remains disputed.[15] Regardless of whether chemotherapy or radiotherapy is employed, patients often encounter a substantial period of immunosuppression lasting 7–14 days. Studies have shown that adoptive cell immunotherapy can restore the host's antitumor immunity, and DC-CIK combined with chemotherapy shows improved clinical efficiency than chemotherapy alone in gastric

Table 2. Summary of treatment-associated adverse events

	Grade 1	Grade 2	Grade 3–4
Fatigue	6 (30%)	0 (0%)	0 (0%)
Nausea	5 (25%)	0 (0%)	0 (0%)
Vomiting	2 (10%)	0 (0%)	0 (0%)
Chills	1 (5%)	0 (0%)	0 (0%)
Fever	5 (25%)	7 (35%)	0 (0%)
Muscle soreness	2 (10%)	0 (0%)	0 (0%)
Skin rash	1 (5%)	0 (0%)	0 (0%)
ALT/AST elevation	2 (10%)	0 (0%)	0 (0%)
Diarrhoea	1 (5%)	0 (0%)	0 (0%)

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

cancer. In the present study, patients with stage III gastric SRCC were infused with NRT after adjuvant chemotherapy. Results indicated that NRT immunotherapy was well tolerated with no occurrence of severe adverse events. Besides, NRT immunotherapy exhibited the potential to reduce recurrence rates in stage III gastric SRCC while simultaneously prolonging survival compared to published literature[6] with an estimated 5-year DFS and OS rate in our study being 44% and 69%, respectively. For those who received chemotherapy alone, the 5-year OS rate was only 29.6% as reported in other studies.[6]

Adoptive cell immunotherapy has exhibited preliminary success against malignancies. Driven by the rapid development in deep sequencing and high-throughput immunoassay technology, mutation-derived neoantigens have emerged as crucial determinants for eliciting effective antitumor immune responses in adoptive T cell therapies.[16] Tran *et al.* identified and amplified a polyclonal $CD8^+$ T cell targeting mutant KRAS G12D in tumor-infiltrating lymphocytes obtained from a patient with metastatic colorectal cancer. Infusion of these specific $CD8^+$ cells leads to the regression of all lung metastases.[17] KRAS G12D targeted T cell-receptor gene therapy was developed based on this research, which remarkably induced substantial regression of metastatic pancreatic cancer.[18]

Currently, the identification of neoantigens largely depends on techniques like whole-exome sequencing (WES) and MHC–peptide binding prediction algorithms. The process is complex and expensive, and also costs a lot of time.[19,20] We have constructed a neoantigen peptide library based on the frequency of mutation of genes in common solid tumors, aiming at identifying mutations in a more timely and straightforward manner.[14] We have successfully identified individualised neoantigens within 20 days based

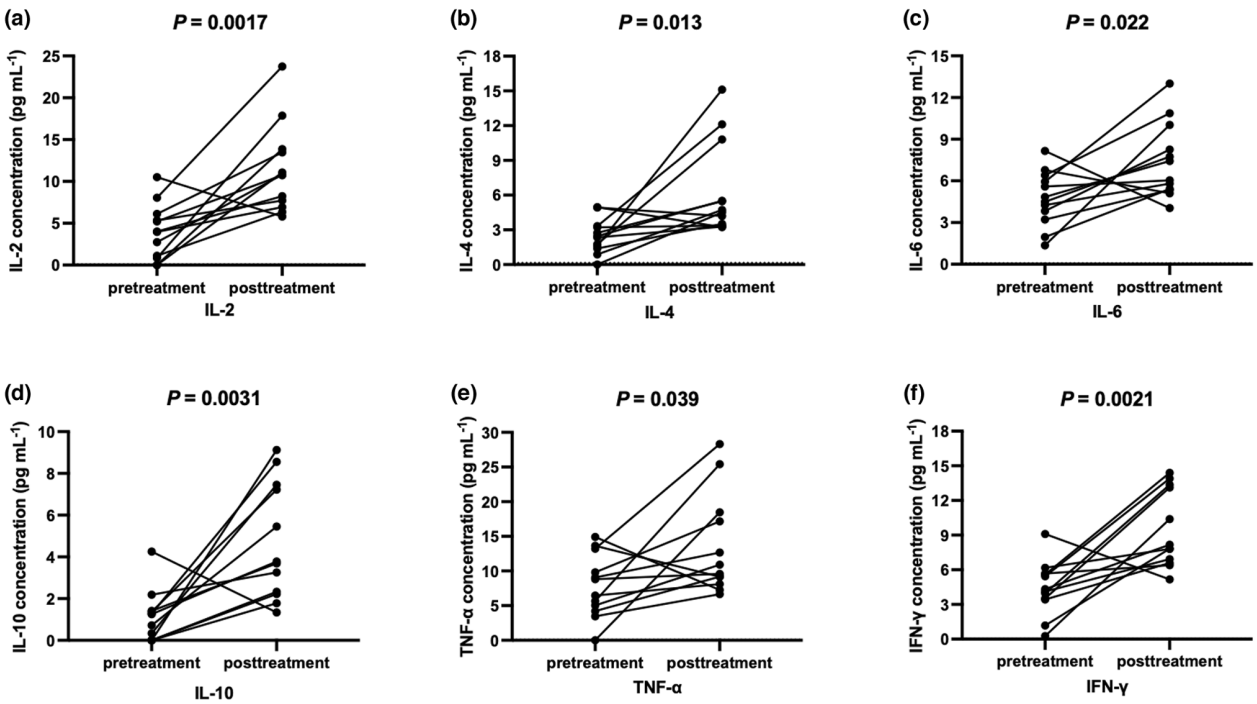

Figure 3. Serum cytokines before and after treatment (n = 12). The serum concentration of IL-2 **(a)**, IL-4 **(b)**, IL-6 **(c)**, IL-10 **(d)**, TNF-α **(e)** and IFN-γ **(f)** were increased after NRT therapy. The dotted line indicates LOD for each assay. Paired Student's t-test.

on preexisting T cell responses, and induced NRTs for adoptive cell immunotherapy. In the present study, one to four candidate neoantigen peptides were chosen for each patient under the established protocol to induce NRTs. After *ex vivo* induction and amplification, the upregulation of CD137 was detected on $CD3^+$ T cells. It is well known that CD137 is not only a co-stimulatory molecular in T cell activation but also a biomarker of tumor-reactive T cells.[16] The result indicated that we successfully induced neoantigen-reactive T cells. The mean proportion of $CD3^+CD137^+$ T cells after *ex vivo* induction is 5.8% (0.52–19.47%), approximate to our previous study. Targeted enrichment and amplification strategy could potentially guide more efficacious *in vivo* antitumor responses. What's more, we could also identify polyclonal T cells targeting neoantigen epitope, laying the foundation for potential TCR-T immunotherapy. We also detected the upregulation of pivotal antitumor cytokines: IL-2, TNF-α and IFN-γ after cell infusion, indicating robust T-cell activation. Pro-inflammatory cytokines such as IL-6 and IL-10 were also elevated.

The study also has some limitations. Firstly, the small sample size is relatively small and without a randomised design. To enhance the study's rigour, a randomised controlled trial with a larger cohort is warranted. Secondly, a crucial challenge in the clinical application of NRT therapy lies in effectively identifying individuals who stand to benefit. Our study suggested that the percentage of $CD3^+$, $CD3^+CD8^+$ and $CD3^+CD4^+$ T cells within infused cells might serve as potential prognostic factors influencing the efficacy of NRT therapy, whereas no statistical difference was observed. Thus, further exploration of more informative immune biomarkers is imperative. Nonetheless, our study established a practical, time-efficient pattern for precision immune cell therapy, which holds the potential for broad application across a spectrum of malignant solid tumors.

In conclusion, we have provided preliminary data showing that NRT is well tolerated, and exhibits promise for eliciting encouraging clinical responses in patients with stage III gastric SRCC.

METHODS

Patients

This study enrolled a cohort of 20 patients who underwent radical surgery with stage III gastric SRCC. All surgical specimens were reviewed by two experienced pathologists. The diagnosis of gastric SRCC was defined by the presence of signet-ring cells in more than 50% of the tumor. Other inclusion criteria were as follows: age of 18–80 years, a projected life expectancy exceeding 3 months, absence of neoadjuvant chemoradiotherapy prior to surgery, satisfactory organ function, and a positive status for the human leucocyte antigens (HLA) A2, A24 or A11. Exclusion criteria included active uncontrolled infection, previously diagnosed other malignancies, pregnancy or nursing, immune deficiency or autoimmune diseases or any condition that could render patients inappropriate for trial participation as judged by their treating clinician.

Study design

This single-centre phase Ib/II study was registered and approved by the Nanjing Drum Tower Hospital Ethics Committee. All patients in this study received postoperative standard docetaxel and S1 or oxaliplatin and S1 chemotherapy. One to four cycles of individualised neoantigen-reactive T cells were infused after the last cycle of chemotherapy. One day before each cycle of T-cell infusion, 250 mg m^{-2} cyclophosphamide was given for lymphodepletion. An average of 8.3×10^9 bulk T cells containing NRT were infused intravenously. Then, 3–5 million IU (MIU) IL-2 were continuously intravenously injected over a total of 5 days (Figure 4). Imaging evaluation was performed every 2–3 months. Patients may discontinue our therapy for refusal, pregnancy or unacceptable toxicity. The duration of DFS was defined as the period from the first treatment to the occurrence of the event (recurrence or death). The OS was defined as the period from initial treatment to death. The primary endpoint of our study was mDFS (median disease-free survival, DFS). The secondary endpoint was safety evaluation and immune response. Adverse events were monitored according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (NCI-CTCAE v3.0).

Selection of personalised neoantigen peptides

Usually, we collected 30 mL of blood to screen for T-cell reactivity before the last cycle of chemotherapy. Individualised neoantigen peptides for the generation of NRT were selected from the inventory-shared neoantigen peptide library, according to the preexisting immunity of the host. Generation and detailed procedures of the catalogued common antigen peptide library have been described in the previous study.[14] The personalised peptides for these 20 patients are described in Table 1.

Generation of neoantigen-reactive T lymphocytes

Neoantigen-reactive T lymphocytes were prepared as previously described.[14] Briefly, PBMCs were collected with COBE Spectra MNC program (Terumo BCT) within 1–2 months after the last cycle of chemotherapy. Then, PBMCs were incubated in AIM-V (Gibco, Invitrogen, CA, USA) medium. Two hours later, non-adherent cells were collected and activated with 100 ng mL^{-1} OKT-3 (eBioscience, CA, USA) and

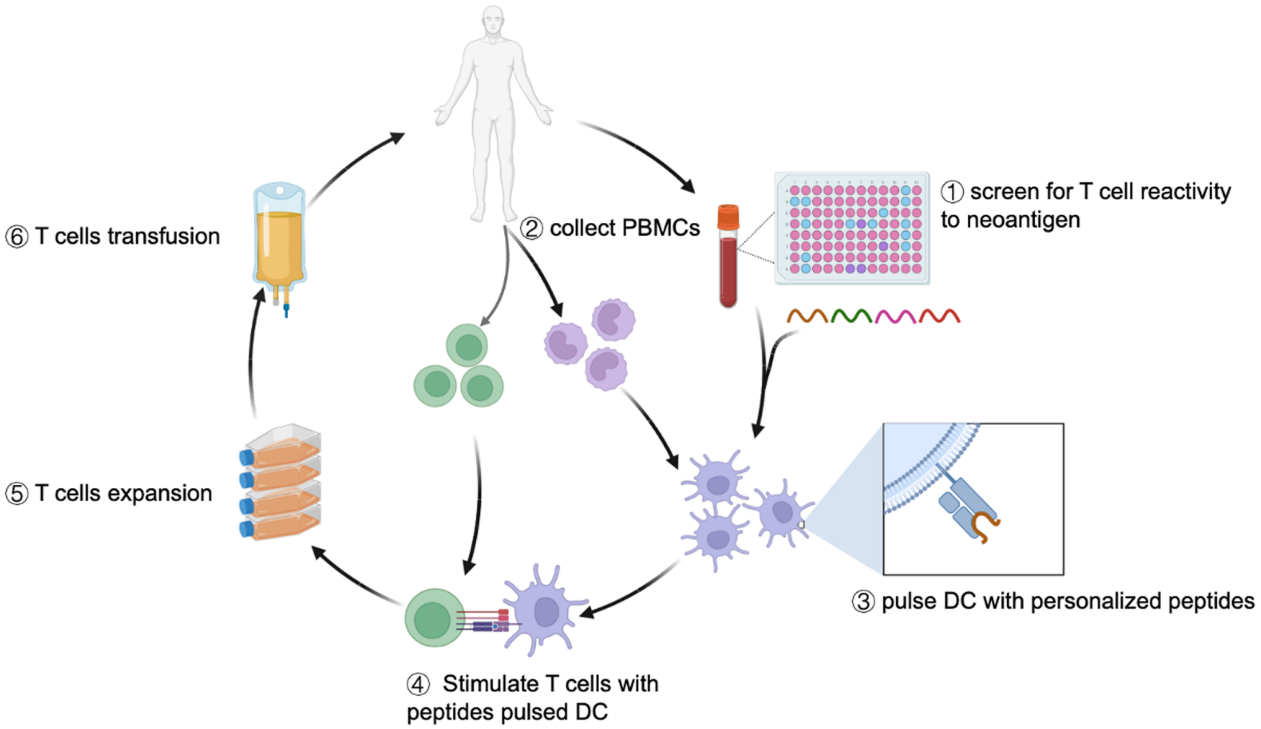

Figure 4. Schema for treatment.

300 U mL^{-1} hIL-2 (eBioscience). To obtain immature DCs, adherent cells were cultured in AIM-V medium (Gibco) containing 800 IU mL^{-1} GM-CSF (PeproTech, NJ, USA) and 1000 IU mL^{-1} IL-4 (PeproTech). On day 5, fresh medium containing 1% HS, 10 ng mL^{-1} LPS (Sigma-Aldrich, MO, USA) and 100 IU mL^{-1} IFN-γ (PeproTech) was added to the culture to obtain mature DCs. Mature DCs were then pulsed individually with identified peptides (10 μM) for approximately 4–6 h. To produce neoantigen-reactive T cells, peptide-pulsed DCs were co-cultured with activated T cells at a ratio of 1:5 to 1:10 in full AIM-V medium supplemented with 5% HS, 100 U mL^{-1} IL-2, 10 ng mL^{-1} IL-7 and 10 ng mL^{-1} IL-15 for 7–10 days.

Phenotypic analysis of peripheral blood immune cell

We performed flow cytometry analysis before and after cell culture using antibodies specific to CD3, CD8, CD4, CD56, CD16, CD19, CD279, CD137, CD45RO and CD62L (BD Biosciences, CA, USA). Antibodies were conjugated with FITC, PE, PerCP-cy5.5 or APC fluorochromes. Before analysis, all samples were washed twice and resuspended in FACS buffer containing indicated antibodies for 30 min at 4°C. Analyses were performed with BD Accuri C6 (BD Biosciences).

Measurement of serum cytokines

The concentration of serum cytokines (interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor-a (TNF-α) and interferon-γ (IFN-γ)) were measured at baseline and before the fourth cycle of cell infusion using cytometric bead array (CBA) kits (BD Biosciences). Samples were analysed with BD Accuri C6 (BD Biosciences).

Statistical analysis

The paired Student's *t*-test was used to analyse the statistical differences in cell phenotype and cytokine level prior to blood collection and after cell infusion. The Kaplan–Meier method was used to estimate the cumulative survival curve. All data are presented as mean ± SEM, and $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS, version 26.0.

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AUTHOR CONTRIBUTIONS

Naiqing Ding: Formal analysis; writing – original draft. **Qin Liu:** Investigation. **Juan Du:** Investigation. **Jie Shao:** Data curation. **Yang Yang:** Investigation. **Ju Yang:** Investigation. **Fangjun Chen:** Formal analysis. **Lixia Yu:** Data curation. **Baorui Liu:** Conceptualization; supervision. **Jia Wei:** Project administration; validation; writing – review and editing.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data will be made available by the corresponding author upon reasonable request.

ETHICS STATEMENT

The study was approved by the institutional review board (CWO) of Nanjing Drum Tower Hospital. All patients provided written informed consent. The Registration No. of the trial is ChiCTR-OIC-17011913.

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